EXHIBIT A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): P. Marraccini et al.
Appl. No.: 10/804,096
Conf. No.: 7981
Filed: March 19, 2004
Title: COFFEE PLANT WITH REDUCED α-D-GALACTOSIDASE ACTIVITY
Art Unit: 1638
Examiner: B.M. Koroma
Docket No.: 112701-535

AFFIDAVIT UNDER 37 C.F.R. § 1.132

Sir:

I hereby state as follows:

1. My experience and qualifications are as follows:

a) BSc. (U. College Galway, Ireland) and MS degrees in Microbiology (N.C. State)
b) PhD Degree in Genetics (U. of Connecticut)
c) 16 year of experience in Molecular Biology and Biochemistry Research
d) 9 years of experience in Plant Biochemistry
e) 7 years of experience in Plant Molecular Biology

2. I have reviewed the outstanding Office Action dated January 27, 2006 pending against the above-identified patent application. As one having ordinary skill in the art, I believe that Patent Office's rejection of Claims 1-7 and 12-14 under 35 U.S.C. §112, first paragraph, is incorrect and based on a misunderstanding of the pending claims. The basis for my opinion is set forth below.

3. The claimed invention of the above-identified patent application relates, in part, to a coffee plant cell that produces galacto-mannans and that is modified to reduce endogenous levels of α-D-galactosidase activity in order to increase galactose branching of the galacto-

mannans. For example, the coffee plant cell can be produced using conventional antisense technology.

4. As one having ordinary skill in the art, I believe that it is within the capabilities of one having ordinary skill in the art to make modified plant cells that produce galacto-mannans and have reduced endogenous levels of α-D-galactosidase activity using antisense technology without undue experimentation in view of the specification. Previous research has provided evidence for the correlation between the reduction of endogenous levels of α-D-galactosidase activity and the increased level of galactose units on the mannan chain. In some plants, the degree of galactose branching on the mannan chains has been found to partially depend on the activity of the α-D-galactosidase (EC 3.2.1.22). It has been discovered that this enzyme is capable of releasing α-1,6-linked galactose units from galactomannans stored in plant seed storage tissue or maturation (Buckeridge and Dietrich, Plant Sci. 117 (1996), 33-43). In addition, the accumulation of galactomannans having a very low galactose/mannose ratio in some plant endosperms or cotyledon tissues has been shown to correlate to peak α-D-galactosidase activity during maturation of these tissues and to the hardening and drying thereof (Kontos and Spyropoulos, Plant Physiol. Biochem. 34 (1996), 787-793). Further, α-D-galatosidases activity has also been associated with the capacity to remove galactose residues, i.e., α-1,6-linked to galactomannan polysaccharides, which brings about a decreased solubility of these polymers (McCleary, Carb. Res. 92 (1981), 269-285). As was previously known, galactose branching on coffee grain mannans decreases from approximately 40% in young grains to the low level found in the mature grains during maturation, which corresponds with α-D-galactosidase enzyme activity increasing during coffee grain maturation.

5. The reduction of endogenous levels of α-D-galactosidase activity (e.g. to increase galactose branching) may be achieved by antisense methods currently known by the skilled artisan. Such a reduced endogenous level of α-D-galactosidase activity can be obtained, for example, by introducing a construct into a coffee plant cell containing a nucleic acid that is transcribed into an antisense copy of the mRNA encoded by the α.-D-galactosidase gene, or to a part thereof. The specification teaches that Applicants found that such an antisense construct

was at least expressed confirming the presence of the α-galactosidase antisense mRNA in coffee embryos derived from transformed plants. Recent data for grain (beans) from the transformed plants indicates that the targeted α-galactosidase gene does have reduced expression in transformed plants (see one example in Exhibit B).

6. As understood by one having ordinary skill in the art, the antisense copy of the mRNA encoded by the α-D-galactosidase gene may be any ribonucleic acid capable of forming dimers under physiological conditions, i.e., to hybridize with the mRNA encoded by the α-D-galactosidase gene under conditions prevailing in the cell. Thus, the antisense copy does not need to be a 100% homologue to the corresponding counterpart, but rather needs to provide sufficient binding to form a dimer. Consequently, antisense copies (and the corresponding nucleic acids from which they are transcribed), that are modified by substitution, deletion and/or insertion of nucleotides are well within the scope of the present invention. Further, the antisense copy may represent a full counterpart to the mRNA encoded by the α-D-galactosidase gene, that is, it may provide a RNA molecule having essentially the same length as the mRNA encoding the α-D-galactosidase polypeptide. On the other hand the antisense copy may only cover a part of the mRNA encoding the α -D-galactosidase polypeptide.

7. Antisense is a widely used technique in plants. When the technique is used, a few different types of constructs can be generated and tested together. Even using the same sequence with the same plasmid may not achieve the same results if the transformation is done differently. Overall, this technique has some intrinsic measure of experimentation. Even in expert hands with a known sequence, the results are variable and need replication. In a majority of cases, 2-4 constructs are made and tested. This does not constitute trial and error in the art of antisense, but is routine to one having ordinary skill in the art. In most cases the full cDNA sequence in antisense orientation will work. It is just a matter of experimental optimization to achieve reduced gene expression. Further, as one having ordinary skill in the art understands, small parts of the cDNA can also work.

8. Barring a currently unanticipated reason, the antisense sequence and the construct described in the specification should work to reduce the expression of the α-D-galactosidase gene in the coffee grain as the whole gene sequence is used. Further, evidence in the specification indicates the construct working properly, and Applicants' recent experimental evidence with beans confirms it is working as expected thereby providing a reasonable expectation of success for the claimed invention.

9. As one having ordinary skill in the art, I find that the specification adequately teaches the skilled artisan how generate modified plant cells that produce galacto-mannans and have reduced endogenous levels of α-D-galactosidase activity. For example, the specification provides adequate disclosure to one of ordinary skill in the art regarding the modification of the coffee plant cell so as to reduce α-D-galactosidase activity using antisense oligonucleotides, thereby producing increased galactose branching of galactomannans and increased water solubility. The antisense oligonucleotides may have of any suitable length or sequence. Moreover, the skilled artisan can use the alpha gal sequence provided by the Applicants to make an antisense construct that is capable of reducing the level of α-D-galactosidase activity in a transformed plant, either in the whole plant by using a ubiquitously expressed 35S promoter to drive the alpha gal antisense gene, or, in the endosperm of the coffee grain (grain specific reduction of expression), by using an 11S promoter as described in the specification. The skilled artisan would be able to further determine similar antisense oligonucleotides that achieve the same a reduction in the level of α-D-galactosidase activity through routine experimentation.

10. One having ordinary skill in the art would also understand that the specification discloses that, if the endogenous levels of α-D-galactosidase activity was reduced, it is possible that the level of galactose units on the mannan chain could be increased (as shown by the previously cited research), which could increase solubility and extractability. For example, as stated in the specification, in coffee grains, cell wall polysaccharides account for approximately 48% of mature coffee bean dry weight, and of these, mannans represent approximately half. These polysaccharides are essentially insoluble in purified form and have very low galactose branching (Bradbury and Haliday, J. agric. Food Chem. 38 (1990), 389-392). Mannan polymers

are acknowledged to be the main reason for the large losses of original green coffee weight encountered during preparation of soluble coffee drinks. The losses occur either when insoluble material remains as sediments during initial extraction or when precipitates and gels form during storage of coffee liquors. Mannans have also been shown to be the principal component responsible for cloudiness and precipitation during standing of coffee beverages. In view of this, even a small relatively effective antisense nucleic acid could be sufficient to give a commercially relevant result in accordance with the present claims.

11. For all the foregoing reasons, as one having ordinary skill in the art, I believe that any skilled artisan can make modified plant cells that produce galacto-mannans and have reduced endogenous levels of α-D-galactosidase activity using antisense technology without undue experimentation in view of the specification. In addition, I believe that modified plant cells that produce galacto-mannans and have reduced endogenous levels of α-D-galactosidase activity are sufficiently described in the specification to convey to the skilled artisan that the Applicants had possession of the claimed invention at the time the application was filed.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this patent and any patent issuing therefrom.

Date: April 7 2006

Print Name: JAMES Mc CARTHY

EXHIBIT B

Exhibit B

Panel A: This shows a section of the RNA blot with the main ribosomal RNA bands and demonstrates that a relatively equivalent amount of total RNA was loaded in the different lanes. This panel also indicates that the transfer of the RNA from the gel to the blot was correctly performed. Panel B: This shows the results obtained after a hybridisation experiment between the RNA shown in Panel A with a P^{32} radiolabelled probe for the alpha-galactosidase cDNA. Lane 1; total RNA from a well characterized transformed plant that was produced by agrobacterium mediated transformation with an anti-sense construct for the coffee alpha-galactosidase gene (such as that described in this patent application). Lane 2; total RNA isolated from a corresponding control plant that was not transformed. The absence of a band corresponding to the alpha-galactosidase transcripts in Panel B lane 1 shows that the level of transcripts for this gene were significantly reduced in the transgenic plant with the anti-sense construct for the coffee alpha-galactosidase gene (transcripts not detected in this exposure). In contrast, the transcripts for the alpha-galactosidase gene were easily detected in the control plant Lane 2.